Nezara viridula (Hemiptera: Pentatomidae) Feeding Patterns in Macadamia Nut in Hawaii: Nut Maturity and Cultivar Effects

PETER A. FOLLETT,[1,2] MARK G. WRIGHT,[3] AND MARY GOLDEN[1,3]

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ABSTRACT *Nezara viridula* L. (Hemiptera: Pentatomidae) is a serious pest of macadamia nuts, *Macadamia integrifolia*, in Hawaii. Using ruthenium red dye to stain stink bug feeding probes, feeding activity was determined for nuts of various maturity levels harvested from the tree and off the ground throughout the growing season in five commercial cultivars. Damage occurred in the tree and on the ground during all nut growth stages. Damage on the ground was often higher than in the tree. Cultivar 246 was more susceptible to attack than cultivars 333 and 800. It was previously thought that cultivar susceptibility was related to husk and shell thickness, but cultivar 246 showed higher damage than other cultivars even during early nut development when the nuts are small and before the shell has formed. This suggests that shell and husk thickness may play a secondary role in susceptibility to feeding by *N. viridula.* Monitoring *N. viridula* feeding activity during early nut development may help alert growers to potential problems later in the season, but early-season probing activity in immature nuts was not a good predictor of damage levels in mature nuts later in the season in our study.

KEY WORDS southern green stink bug, ruthenium red dye, integrated pest management

Macadamia nut (*Macadamia integrifolia,* Proteaceae) is the largest orchard crop grown in Hawaii, with 18,000 acres in production and a total farm value of \$30–40 million (HASS 2008). *Nezara viridula* L. (Hemiptera: Pentatomidae) is one of the main insect pests of macadamia nuts. Although damage is typically low (<2%), some years *N. viridula* populations increase to outbreak levels (25–50% damaged nuts) for unknown reasons (Mitchell et al. 1965, Jones and Caprio 1992, Wright et al. 2007). *N. viridula* causes direct damage by inserting its mouthparts through the husk and shell to feed on the macadamia nut kernel and will feed on nuts of all sizes in the field (Golden et al. 2006). *N. viridula* feeding also causes premature drop resulting in immature nuts falling among mature nuts that will later be harvested from the ground (Jones and Caprio 1994) and may introduce pathogens resulting in moldy nuts, both of which result in rejection at the processor. Immaturity and pathogens may raise actual annual losses to this stink bug (Jones and Caprio 1994). *N. viridula* is the only heteropteran known to feed extensively on macadamia nuts in Hawaii (Jones 2002).

Historically, most information on *N. viridula* and other insect damage to macadamia nuts came from laboratory analysis of harvest samples of mature nuts at the processor. The problem with using crop loss assessment information from the processor is the lag time—stink bug populations may be increasing and damaging nuts 3–4 mo before harvesting begins, and information is usually not available from processor samples until a month after the nuts are harvested. Therefore, the information comes after the damage occurs and the grower has no chance to react to, or preempt, an outbreak.

A key element to any stink bug integrated pest management (IPM) program is the ability to monitor population levels so that outbreaks can be anticipated and management decisions made in a timely fashion (Wright et al. 2007). Traps and pheromones are often used to monitor pest population trends, but there are currently no effective traps or lures to catch *N. viridula.* As an alternative means of monitoring stink bug activity in macadamia nuts, a technique using ruthenium red dye was developed to stain stink bug probes (Golden et al. 2006). The dye will stain stink bug feeding probes in both immature and mature nuts, allowing evaluation of stink bug feeding activity and providing an indirect indication of population trends throughout the year. Staining of probes in the husk is highly correlated with kernel damage, and evaluation of damage using the dye accurately reflects damage levels determined at the processor (Golden et al. 2006).

Macadamia trees have indeterminate flowering, and the time from anthesis to nut drop is ≈30 wk (Nagao and Hirae 1992). The main period of harvest is August through January (multiple harvests), but stink bug feeding may occur throughout the year on either developing nuts or mature nuts (Jones and Caprio 1992, Golden et al. 2006). Macadamia is not a preferred food

[1] USDA–ARS, U.S. Pacific Basin Agricultural Research Center, PO Box 4459, Hilo, HI 96720.

[2] Corresponding author, e-mail: pfollett@pbarc.ars.usda.gov.

[3] Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822.

plant for *N. viridula* (Shearer and Jones 1996). *N. viridula* feeds on leguminous and cruciferous weeds in macadamia orchards and moves on to macadamia nuts in the tree and on the ground when weeds begin senescing (Jones et al. 2001) or are controlled by mowing or herbicide use (Jones 2002).

Previous research using the dye to track stink bug feeding activity used orchard blocks as the sampling universe (Golden et al. 2006), without accounting for differences in damage by cultivar. Macadamia nut orchards in Hawaii are typically made up of 5–10 different cultivars that are interspersed, but usually orchard blocks consist of one or a few main cultivars. Certain cultivars such as 246, 344, and 508 are believed to be more susceptible to *N. viridula* damage, whereas the cultivars 333 and 800 are believed to be less susceptible (Jones and Caprio 1992, Jones 2002). To effectively implement integrated management of *N. viridula* in macadamia orchards, it is important to predict when damage is occurring and which cultivars are most susceptible so that targeted remedial measures can be taken if needed. Studies were conducted to determine cultivar susceptibility throughout the growing season in nuts of varying maturity classes using ruthenium red dye to stain stink bug probes. Data were also taken on cultivar husk and shell thickness of mature nuts, and *N. viridula* proboscis length, to infer cultivar susceptibility.

Materials and Methods

Proboscis Length and Shell and Husk Thickness. Macadamia nuts and various *N. viridula* life stages were collected from multiple orchards in two distinct growing areas of the Big Island of Hawaii, North Kohala district (average rainfall 94 cm/yr; thick vegetation between tree rows) and Kau District (average rainfall 61 cm/yr; sparse vegetation between tree rows) to develop a nut maturity classification system, to measure nut size in various cultivars, and to measure proboscis length in each *N. viridula* life stage. Nut measurements for the classification system included nuts of several cultivars grouped together. Class 1 nuts averaged 16.5 mm in diameter at 8 wk from anthesis (full flowering). A class 1 nut has a very soft shell, the kernel is immature, and the husk is adhering to the shell. Class 2 nuts averaged 18.6 mm in diameter at 12 wk from anthesis. A class 2 nut still has a soft shell and an immature kernel, but there is separation of the husk from the shell. Class 3 nuts averaged 26.5 mm in diameter, at 18 wk from anthesis. A class 3 nut had a hardened shell, a mature kernel, and white to tan husk interior. Class 4 nuts averaged 31.6 mm in diameter at 24 wk from anthesis. A class 4 nut has a hard shell and fully mature kernel, and the husk interior is light brown to chocolate brown.

Macadamia nuts of various cultivars were collected at three times during a 2-yr period to measure nut size of mature (class 4) nuts ($n = 1{,}100$). Nuts were cut in half, using a band saw, along the equator equidistant from the stem and flower ends. Measurements were made of the nut diameter, and the minimum and maximum thickness of the shell and husk using a hand held micrometer. Wild *N. viridula* nymphs and adults were collected at various times during the year (adults, $n = 150$; immature stages, $n = 25$–30), and their proboscis lengths were measured using a stereomicroscope and ocular micrometer.

Ruthenium Red Dye Technique. Ruthenium red dye was used to stain *N. viridula* probes and evaluate feeding activity. To stain feeding probes, freshly harvested nuts were submerged in a 0.05% aqueous solution of ruthenium red in deionized water, shaken for 2 h at room temperature (23 ± 2°C), and rinsed in distilled water for 30 min (Golden et al. 2006). Stained probes ($\approx$0.50 mm diameter) on the surface of the shell were visualized under a stereomicroscope ($\times$6) as a circular, magenta-pink spot. Stained probes on the inside of the shell and husk, and on the kernel, appear as discrete pink spots or somewhat larger circular light pink areas (see color photos in Golden et al. 2006).

Comparison of Commercial Harvest Methods. Ten trees were selected for each of the cultivars 246, 333, 344, 508, and 800 to compare *N. viridula* damage in nuts from commercial shaker and ground harvests at MacFarms of Hawaii (Captain Cook, HI; Kau District). During shaker harvesting in November 2004, 50 mature nuts (class 4) were collected from the ground before shaking and from the catch frame after shaking for each individual tree and evaluated for damage. Nuts collected from the ground were brown, suggesting they had fallen >2 wk before collection. *N. viridula* prefers to feed on green nuts on the ground, so once the nuts have turned brown, additional damage is minimal (Jones and Caprio 1994). Nuts were dyed and probes to the kernel were recorded.

Seasonal Pattern of Damage. Immature and mature nuts from various cultivars grown at Island Harvest (Kapaau, HI; North Kohala district) and MacFarms orchards were sampled throughout the period of nut development to determine *N. viridula* feeding activity using the dye method as described above. Ten trees each of the cultivars 246, 344, and 508 at Island Harvest and cultivars 246, 333, and 800 at MacFarms were randomly selected and evaluated at 2-mo intervals. Beginning in February, 50 nuts of the prevalent maturity class were collected from the tree and from the ground, dyed, and examined for stink bug feeding on the kernel.

Statistical Analysis. Shell and husk thickness and nut diameter data for each cultivar were analyzed using single factor analysis of variance (ANOVA) while blocking for collection date. Mean separations were done using a Tukey's test ($P < 0.05$). Percent damaged kernel data from the commercial harvest methods study were arcsine transformed and subjected to ANOVA, and means separations were done using a Tukey's test ($P < 0.05$). For the seasonal pattern of damage study, data on kernel damage were analyzed separately for each growing area as a factorial design; percent damaged kernels data from this study were arcsine transformed and analyzed using a three-way ANOVA, with date (nut maturity level), cultivar, and nut collection type (ground or tree) as sources of variation. Because there was a significant three-way interaction effect for date and cultivar and harvest method for MacFarms data, the effect of harvest method for each cultivar on each date was examined in separate one-factor analyses for both farms. Means separations were done using a Tukey's test (SAS Institute 2002).

Results

Proboscis Length and Shell and Husk Thickness. Various *N. viridula* life stages are easily differentiated by size (Follett et al. 2007). Mean proboscis lengths for *N. viridula* life stages were 0.9 (first instar), 1.9 (second), 3.8 (third), 4.7 (fourth), 5.2 (fifth instar), and 6.7 (adult) mm. Proboscis length for each *N. viridula* life stage was significantly different from the other life stages ($P < 0.05$), and the overlap between the maximum proboscis length of one stage and the minimum length of the next stage was minimal or nil (Fig. 1).

Mean diameter of mature nuts was 33.2, 33.2, 32.8, 29.8, and 29.7 mm for cultivars 246, 800, 344, 333, and 508, respectively. Diameter measurements include the husk, shell, and kernel. Cultivars 246, 800, and 344 were significantly larger than cultivars 333 and 508 (Tukey's test, $P < 0.05$). Minimum husk and shell thickness and maximum husk and shell thickness varied significantly among cultivars ($P < 0.001$). Cultivar 800 had a significantly greater mean minimum husk thickness than the other cultivars, and cultivars 246 and 333 had a significantly greater minimum husk thickness than cultivars 344 and 508 ($P < 0.05$). Cultivars 800 and 246 had a significantly greater mean maximum husk thickness than cultivars 333, 344, and 508 ($P < 0.05$; Fig. 1).

Results suggest that all macadamia nut varieties grown in Hawaii are potentially susceptible to adult stink bug probing to the kernel of mature nuts (Fig. 1). The mean proboscis length for adults was 6.5 mm, and the longest proboscis' measured was 7.5 mm. The shortest adult proboscis was 5.4 mm, which was greater than the average minimum shell and husk thickness of the thickest cultivar (800, 5.3 mm). Even a large *N. viridula* adult with a long proboscis may not be able to probe to the kernel at the thickest point of the nut, particularly for cultivars 246 and 800 (Fig. 1). Fifth instars are capable of injuring a smaller fraction of nuts than adults because of a shorter proboscis, and instars 1–4 probably cannot injure the kernels of full-size mature nuts at all (Fig. 1).

Comparison of Commercial Harvest Methods. The comparison of stink bug damage between mechanical tree shaking and hand picking nuts from the ground was not significant ($P = 0.61$) for the six cultivars tested (Table 1). Overall kernel damage (nuts shaken from the tree + nuts off the ground) was significantly different among cultivars. Cultivars 344, 741, 508, and 246 were generally more damaged by *N. viridula*, whereas cultivars 333 and 800 were less damaged. Cultivar 344 showed the highest mean kernel damage (10.0%), and cultivar 800 showed the lowest (1.3%; Table 1).

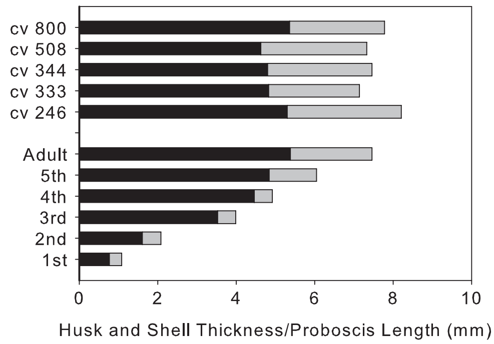

Fig. 1. Comparison of average minimum (black) and maximum (gray) husk and shell thicknesses (probe distance to the kernel) for each cultivar and minimum (black) and maximum (gray) proboscis lengths of different life stages of *N. viridula*.

Seasonal Pattern of Damage. In the seasonal pattern of kernel damage experiments, probing of nuts was observed in all cultivars on nearly all dates at both farms (Figs. 2 and 3). At MacFarms, results were highly significant ($P < 0.001$) for the effects of date, cultivar, and nut position (from the tree or off the ground) and for all the interaction effects, date by cultivar, date by position, cultivar by position, and date by cultivar by position. Cultivar 246 was significantly ($P < 0.05$) more damaged than cultivars 333 and 800, and damage to nuts on the ground had significantly higher damage ($P < 0.05$) than nuts collected from the tree (Fig. 2). The highest probing activity was observed in cultivar 246 nuts early during development; 30% of class 1 nuts off the ground in February showed evidence of probing, and 2 mo later, 15 and 10% of class 2 nuts collected off the ground and from the tree were probed (Fig. 2). Probing of cultivars 333 and 800 nuts remained <8% during the same period. Probing of mature (class 4) nuts during the period from August through December was similar for cultivars 246, 333, and 800, averaging 3.2, 2.4, and 1.1%, respectively.

Table 1. Mean percent kernel damage (±SEM) for six cultivars of macadamia nuts harvested by mechanical tree shaking or by hand from the ground (MacFarms)

Cultivar	Harvest Method		Overall
	Shaker	Ground	
344	8.8 (2.6)	11.2 (2.2)	10.0 (1.7)a
741	6.4 (1.2)	7.8 (1.7)	7.1 (1.0)a
508	5.8 (1.5)	8.0 (1.8)	6.9 (1.2)a
246	4.2 (1.3)	4.8 (1.3)	4.5 (0.9)ab
333	1.8 (0.7)	1.8 (0.5)	1.8 (0.4)bc
800	1.0 (0.4)	1.6 (0.6)	1.3 (0.4)c

Cultivars are sorted by decreasing overall damage. ANOVA on harvest method was not significant ($P = 0.61$). Overall cultivar means (±SEM) within a column followed by different letters were significantly different by a Tukey's test ($P \leq 0.05$).

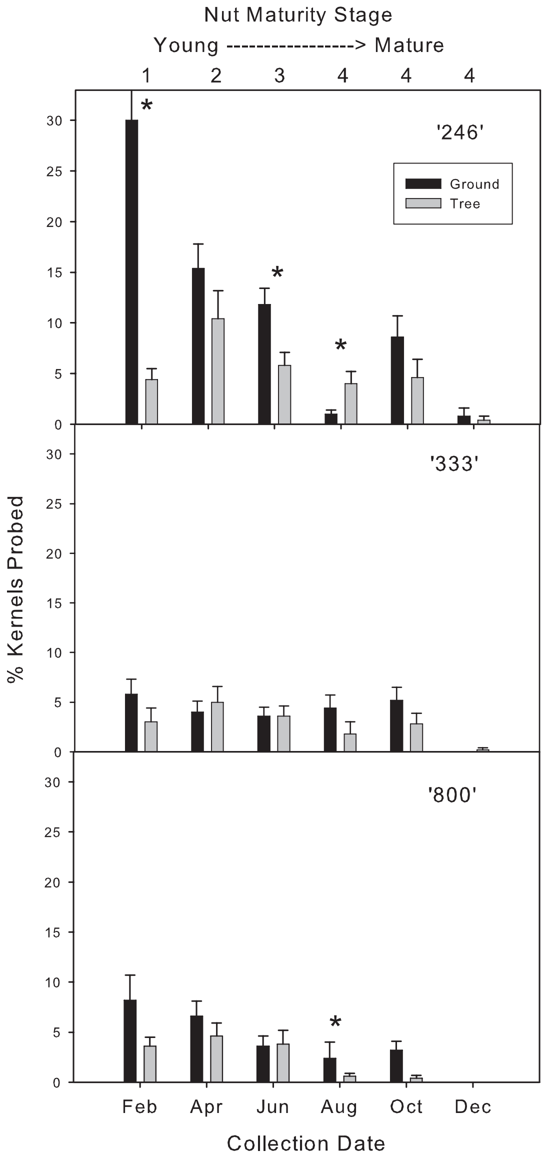

Fig. 2. Seasonal damage patterns for cultivar 246, 333, and 800 nuts of various maturity levels collected from the tree and off the ground (MacFarms). *Significant treatment effect between nuts collected from the tree and off the ground.

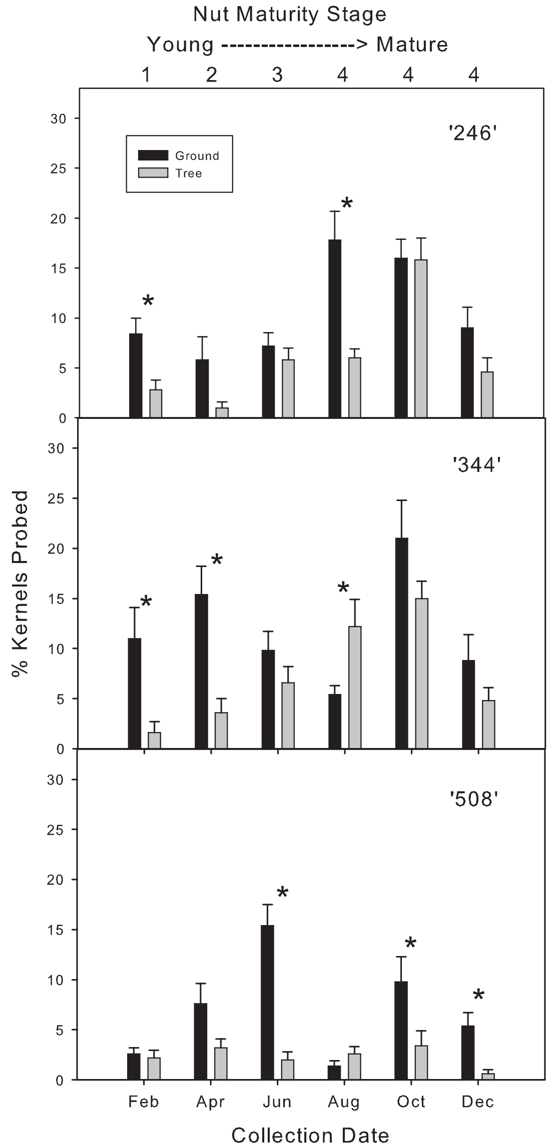

Fig. 3. Seasonal damage patterns for cultivar 246, 344, and 508 nuts of various maturity levels collected from the tree and off the ground (Island Harvest). *Significant treatment effect between nuts collected from the tree and off the ground.

At Island Harvest, results were significant ($P < 0.02$) for the effects of date, cultivar, date by cultivar, and nut position. Cultivar 508 was significantly ($P < 0.05$) less damaged than cultivars 246 and 344, and damage in nuts off the ground was significantly higher ($P < 0.05$) than nuts collected from the tree (Fig. 3). The highest probing activity was observed in mature nuts collected off the ground in cultivar 344 in October (21.0%). Probing of mature (class 4) nuts during the period from August through December was considerably higher at Island Harvest than at MacFarms, with cultivars 246, 344, and 508 averaging 11.5, 11.2, and 3.9%, respectively.

Linear regressions were done on the percent probed immature nuts by *N. viridula* against the numbers of days since the start of sampling (three collections at 0, 60, and 120 d). Slopes of the regression lines were significantly different from zero for only 3 of the 12 comparisons (Table 2); slopes for nine regressions did not differ significantly from zero. For cultivar 246, the percent probed nuts on the ground decreased at MacFarms but did not change significantly at Island Harvest; the percent probed nuts from the tree at Island Harvest and MacFarms did not change significantly during the 120-d period. Percent probed nut

Table 2. Regression analysis of *N. virdula* probing activity (% kernels probed) against time during the season for immature nuts sampled off the ground and from the tree

Cultivar	Location	Intercept ± SE	Slope ± SE	R^2	P
MacFarms					
246	Ground	28.2 ± 2.6	−0.15 ± 0.3	0.42	0.001[a]
	Tree	6.2 ± 1.9	0.01 ± 0.02	0.008	0.63
333	Ground	5.6 ± 1.1	−0.02 ± 0.01	0.06	0.20
	Tree	3.6 ± 1.2	0.005 ± 0.016	0.003	0.76
800	Ground	8.4 ± 1.6	−0.04 ± 0.02	0.11	0.07
	Tree	3.9 ± 1.1	0.002 ± 0.014	0.001	0.91
Island Harvest					
246	Ground	7.7 ± 1.6	−0.01 ± 0.02	0.01	0.64
	Tree	1.7 ± 1.0	0.03 ± 0.01	0.12	0.06
344	Ground	12.7 ± 2.5	−0.01 ± 0.03	0.004	0.76
	Tree	1.4 ± 1.2	0.04 ± 0.02	0.20	0.01
508	Ground	2.1 ± 1.5	0.11 ± 0.02	0.51	0.001
	Tree	2.6 ± 0.75	−0.001 ± 0.01	0.01	0.86

[a] Analysis included only nuts from first three sampling dates (immature nut stages) before the start of harvesting.

regression lines for nuts collected from the ground trended downward in five of the six cultivar collections (cultivars 246, 333, and 508 at MacFarms; cultivars 246 and 344 at Island Harvest), but was significantly positive in cultivar 508 at Island Harvest. Conversely, percent probed nut regression lines for nuts collected off the tree trended upward in five of the six cultivar collections (cultivars 246, 333, and 508 at MacFarms; cultivars 246 and 344 at Island Harvest), but was negative in cultivar 508 at Island Harvest (Table 2).

Discussion

Cultivar susceptibility at different stages of nut maturity had not previously been reported. In the past, information on cultivar susceptibility has focused on mature nuts because this is the stage that is harvested, and stink bug damage is readily apparent in processed nuts after drying as darkened pits caused by feeding (Jones 2002, Wright et al. 2007). The developing kernels in immature nuts turn dark brown when dried, preventing any assessment of stink bug feeding. Ruthenium red dye stains feeding probes in nuts at any stage of maturity that permits study of feeding activity throughout nut development (Golden et al. 2006).

Levels of insect damage in macadamia nuts are in some cases correlated with shell thickness, and shell thickness varies with cultivar and rainfall. For example, tropical nut borer, *Hypothenemus obscurus* (Coleoptera: Scolytidae), damage decreases with increasing shell thickness, and damage is typically higher for a cultivar in drier areas and in drier years because the shell is thinner (Jones 2002). The relationship between shell thickness and susceptibility to *N. viridula* is less clear. Jones (2002) indicated that cultivar susceptibility in mature nuts was a combination of husk and shell thickness, the tendency of the husk to split, and the stage of the stink bug feeding on the nut. Certain cultivars such as 246, 344, and 508 are clearly more susceptible to *N. viridula* damage than other cultivars such as 333 and 800 (Jones and Caprio 1992, Jones 2002, this study). Our measurements of shell and husk thickness at three times during a 2-yr period showed that cultivars 246 and 800 had the greatest maximum shell thickness and 800 had the greatest minimum shell thickness. Cultivar 800 was the least damaged cultivar at MacFarms but cultivar 246 showed higher damage compared with cultivar 333 with a thinner husk and shell. At Island Harvest, cultivar 246 was as damaged as cultivar 344, which has a thinner husk and shell. Hence, cultivar 246 was relatively susceptible even though it was a larger nut and had a generally thicker husk and shell than several other varieties. Also, our results showed that the relative susceptibility of a cultivar is maintained throughout nut development. For example, at MacFarms, cultivar 246 was more susceptible to attack compared with cultivars 333 and 800 even during early nut development when the nuts are small and before the shell has formed (Fig. 2). Conversely, cultivars 333 and 800 showed low probing activity by *N. viridula* at all maturity stages. This suggests that shell and husk thickness may play a secondary role in susceptibility to feeding by *N. viridula* and that other physical or chemical characteristics of cultivars may be the primary determinants of susceptibility.

Monitoring early season nut damage might be beneficial if it is a good predictor of damage levels later in the season when harvesting begins (Wright et al. 2007). If *N. viridula* is feeding on nuts throughout nut development, the expectation would be that kernel damage would increase over time. However, the pattern of *N. viridula* probing activity during the season was variable for nuts collected from the ground and off the tree for the three cultivars sampled at MacFarms and Island Harvest (Figs. 2 and 3). At MacFarms, kernel damage generally decreased from February to December for cultivars 246 and 800, but damage fluctuated with no clear pattern in cultivar 344. For cultivar 246, probing activity at Island Harvest generally increased during the season, whereas probing activity at MacFarms decreased over the same period. Probing activity in immature nuts trended downward in nuts collected off the ground and upward in nuts off the tree, but few of the trends were significant (Table 2). Therefore, early season probing activity in immature nuts was not a reliable predictor of damage in mature nuts harvested later in the season in our study and would not be a useful sampling target for scouting.

Several factors may cause the observed feeding patterns. *N. viridula* cannot complete development on macadamia nuts alone, and additional nutritional resources, such as leguminous weeds, are required for reproduction (Shearer and Jones 1996). Therefore, *N. viridula* may move to and from macadamia trees as different preferred weed hosts in or near the orchard produce fruiting structures then senesce (Jones et al. 2001). Also, *N. viridula* damage early in the season when nuts are developing results in nut drop, but older nuts rarely drop (Jones and Caprio 1994). Nut drop during early nut development is not considered detrimental to production as macadamia nuts also thin themselves naturally. Natural thinning, nut drop caused by feeding damage and cultivar differences may complicate use of early season feeding activity on immature nuts to predict damage of mature nuts at harvest. Because macadamia nut has indeterminate flowering, all maturity stages of nuts can be found on trees throughout the season in many years. It is not known whether *N. viridula* prefers feeding on certain maturity stages given a choice on the tree, and this topic warrants further study. Wright et al. (2007) showed that early season damage estimates from sampling of mature nuts during the period when most nuts are maturing on the tree could be used to predict late season damage levels. When mature nuts are present on the tree during early season, probing activity to these nuts should be a better predictor of damage levels in mature nuts later in the season at harvest compared with immature nuts.

Acknowledgments

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